Chapter

Self-Healing of Concrete through Ceramic Nanocontainers Loaded with Corrosion Inhibitors and Microorganisms

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Abstract

Self-healing was considered for repairing of destruction of reinforced concrete on bridges, houses, etc., that comes from corrosion of reinforcement metals as well as cracking in cement. The work was accomplished at producing and assessing through incorporate ceramic nanocontainers loaded with microorganisms. We produced various types of organic and inorganic nanocontainers that were incorporated into the cement that can act as carriers for the transport of bacteria. The microorganisms used in the work are *Escherichia coli* and *Staphylococcus aureus*. Precipitation of $CaCO_3$ was observed by both bacteria. As microspheres do not affect the submersion of the mineral by the microorganism, additional studies were carried out to assess the interaction between transmission microsystems and bacterium. The mechanism of self-healing of building materials in this work was based on $CaCO_3$ precipitation, through the ureolytic action of bacteria. When a crack appears in the cement, then the bacterium trapped in a nanocontainers is released and comes into contact with the water. In this way, the microorganism begins to metabolize and precipitate the mineral, in a way that eventually observes healing of the crack. $CaCO_3$ microbial precipitation was based on the breakdown of urea ($CO(NH_2)_2$) into CO_3^{2-} and NH_3. Due to the high pK value of the NH_3/NH_4^+ system (pKa = 9.2), the breakdown reaction led to an increase in pH, favoring the release of carbonated ions (CO_3^{2-}), and in an environment rich in calcium ions (Ca^{2+}), $CaCO_3$ precipitation took place.

Keywords: nanocontainers, self-healing, Concrete, corrosion

1. Introduction

Reinforced concrete is a composite material resulting from the strengthening of concrete with steel rods with greater strength. The combination of these materials leads to a new one that will meet the needs of the construction. The reinforcement is done to increase the tensile strength of concrete. On the other hand, steel rods are sensitive to corrosion. Concrete, though, forms an alkaline environment that hinders the oxidation of steel. There are many factors that affect the strength of concrete and contribute to its early wear. A key cause that activates various wear

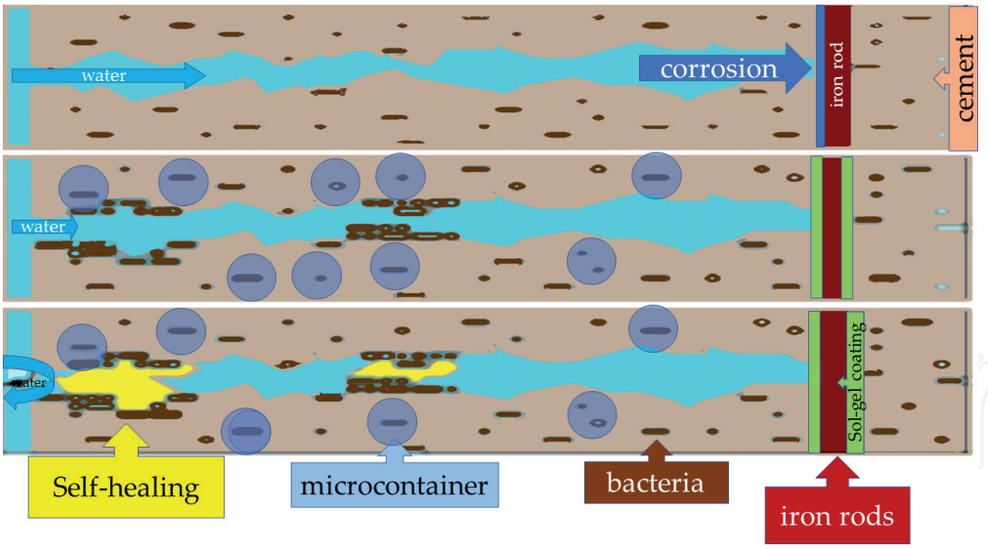

Figure 1.
Schematic representation of the healing of cracks in cement with integrated microorganisms in microcontainers and protective sol-gel coating against corrosion of metal rods.

mechanisms is cracks that dramatically increase the permeability of cement. The microstructure of hardened cement is porous, isolated, and interconnected. The interconnected pores determine the permeability of the material as they allow water and chemical compounds to penetrate the concrete matrix. Permeability essentially increases as the crack connects isolated resources to resource networks. In most concrete wear mechanisms, permeability plays an important role. Specifically, interconnected pores determine the permeability of the material as they allow water and chemical compounds to penetrate the concrete matrix. In the same way, CO_2 diffuses through the pores, which reacts with the alkaline components of cement (e.g. Ca $(OH)_2$) in the process called carbonation.

The above points make it clear that cracks in concrete must be reduced and that ideally a mechanism should lead to the sealing or clogging of newly formed cracks, in order to limit the permeability of the uterus. A healing mechanism is ideal when you do not need constant testing for repair, and it is financially lucrative [1]. In recent times, concrete self-healing is proposed to be done using a biological restoration technique through the introduction of bacteria into the concrete. **Figure 1** presents such technology. The idea is based on the incorporation of a bacterium that metabolizes urea and sinks $CaCO_3$ into the crack environment. $CaCO_3$ microbial submersion is certified by a number of factors, such as the concentration of dissolved inorganic carbonate ions and Ca^{2+} ion concentration. The protection of the bacterium in the cement is done by locking them in micro-containers, the incorporation of which can reduce the strength of the concrete.

The present work deals with the development of coatings to protect steel rods from corrosion and nanocontainers filled with bacteria to induce self-healing.

2. Materials and methods

2.1 Chemicals

The reagents we used are methyl methacrylate (MMA), poly(ethylene glycol) methacrylate (PEG), ammonia solution (30%), 2,2-azobis(2-methylpropionitrile) (AIBN), sodium dodecyl sulphate (SDS), ethylene dimethacrylate (EGDMA),

toluene diisocyanate (TDI), tetraethyl orthosilicate(TEOS), ethylene diamine (EDA), diethylene triamine (DETA), polyvinylpyrrolidone (PVP), chloride ammonium, tryptone, yeast extract, and sodium chloride. The solvents we used were distilled water, acetonitrile, acetone, ethanol, 1-octadecene and paraffin. All chemicals were obtained by commercial sources.

2.2 Instruments

FEI's scanning electronic microscope (SEM) was used with tungsten filament at 25 kV. A Perkin Elmer's FT-IR Spectrum100 spectrometer was used where the scanning range was from 4000 cm^{-1} to 400 cm^{-1}. X-ray diffraction (XRD) was measured using a Siemens D-500 apparatus. The thermal analyses were carried out with Perkin Elmer analyst Pyris Diamant S II. Dynamic light scattering (DLS) was performed at Malvern's Zetasizer Nano and each measurement represents the average of three measurements. A Sorvall Evolution RC centrifuge was used for container separation. An Elma Sonic ultrasonic bath, S 30H, was used to disperse the samples. The sterilizations were carried out with steam sterilizer of Trade Raypa. Any utensils and solutions to be used for the development of microorganisms were sterilized at 120°C for 20 min.

2.3 Nutrient (solutions and LB agar)

The microorganisms used to carry out the experiments were grown in an aqueous culture medium Luria Broth (LB). Recommendation of the culture medium LB was NaCl 0.5% w/v, tryptone 1% w/v, and yeast extract 0.5% w/v. In a Petri dish, nutrient material is placed in which the cells of the microorganisms of the experiments grow. The composition of the nutrient is agar (gel agent) 1.5 g, NaCl (electrolyte) 0.5 g, tryptone (source of proteins and nitrogen) 0.5 g, fungal extract (source of metals and carbohydrates) 0.3 g and distilled water 100 ml. Each dish is partially filled with warm LB agar containing a mixture of substances described in the table above. The investigation of cell vitality is a characteristic indicator and a necessary condition in cases of cytotoxic action of polymer microspheres where they will be used in materials that will come into direct contact with humans. It is performed by identifying vital cell functions. Cell vitality refers to the percentage of healthy cells in a culture. This indicator, by definition unclear, is usually determined by controlling vital functions such as cell metabolic activity (MTT).

2.4 Sterilization of glassware and nutrients

All tools used in microorganism experiments were sterilized to avoid any contamination of microbial solutions. The same procedure was carried out for their nutrients. All glassware and nutrients were sterilized for 20 min at 120°C. For the development of the microorganism, the experimental process of cultivation in nutrient solutions is described as follows: the reculture of the microorganism takes place after recovery from ampoules stored at −80 °C. The nutrient medium is vaccinated with a small amount of the ampoule of the microorganism. The procedure takes place under aseptic conditions, conditions achieved by the use of a reducing flame in order to avoid contamination of the sample. Vaccination of the microorganism is done with a sterile Pasteur pipette, where its nose has been sterilized in flame. The nutrient solution with the microorganism is incubated in a special stirring chamber at 37°C, at approximately 100 rpm for 24 hours in order to optimally develop the microorganism. For the development of the microorganism used each

time, the experimental process of growing it in a Petri dish is as follows: ampoules of the microorganism are inoculated in a Petri dish forming zeta as shown in the following image. The procedure takes place under aseptic conditions using a reducing flame to avoid any complications of the sample. The microorganism is vaccinated in the dish with sterile Pasteur pipette, where its nose has been sterilized as above. The vaccinated dish is then placed in an incubation oven at 37°C for about 24 hours.

2.5 Cell cultures

The type of cells that covered the needs of this work are THE-293. They're human cells of fetal tissue. For their maintenance they contain adenovirus and therefore their management must be very careful. They are used for efficacy tests, host contamination tests and iodide tests [2]. Their development conditions depend on the specific characteristics of the cells. Cell culture materials consist of a nutrient cell growth solution DMEM (Dulbecco's Modified Eagle Medium) enriched with 2 mM glutamine, 0.85 g/L NaHCO3, 25 mM HEPES, 10% FBS (Fetal Bovine Serum), 6.8 < pH < 7.2. 0.2% w/v streptomycin, 2 × 103 U/mL penicillin, in PBS (1×) and cell separation solution (0.02% EDTA-thrypsin). Cell culture is suspended with fresh nutrient solution, at a final concentration of 2–4 × 10^5 cells/mL, and maintained at 37°C, removal of nutrients from cultivation, addition of cell separation solution (4 mL/bottle 75 mL) and incubation at 37°C for 4 min, removal of the solution, re-addition of 0.4 mL of cell separation solution and incubation at 37 °C for 15–20 min, in order to detach the liver and separate the cells and add a DMEM nutrient solution and cell growth at 37 °C. Cell lines can undergo uncontrolled changes related to their morphology, growth, vitality, and karyotype due to prolonged recultures or any unfortunate infections. This risk is avoided by creating a renewable cell bank, after cooling the cells and keeping them in liquid nitrogen for long periods of time (years). Cooling is led to cells that are in a logarithmic phase of growth or are close to filling a single carpet. The methodology followed is (a) cell implantation at a concentration of 4 × 10^5 cells/mL, (b) at 48 h, where the cells are at the completion of the logarithmic growth phase; detachment and centrifugation at 1000 rpm for 5 min, (c) re-dissolution of cells in DMEM in the presence of 10% DMSO in FBS (cryoprotective substance), at a final concentration of 4 × 106–10 × 10^6 cells/ mL (depending on the cell line), (d) transfer to ampoules which are then gradually placed in a freezer; so as to avoid the creation of crystals inside the cells and therefore cellular solution, and (e) finally is placed in a cell retention device (–196 °C, liquid nitrogen) for several years. The restoration of cells stored in liquid nitrogen into current culture (36.5–37°C) is carried out by quickly defrosting the ampoule sample at 37°C (avoid denaturing the protein content of the cell). Centrifuge to remove cold protective material (DMSO) from cultivation. Rapid removal of DMSO is particularly important because it acts as an inhibitor in the development of cell proliferation and in some cases triggers differentiation, apoptosis, or even necrosis; depending on the cell type, redialysis of cells in nutrient solution and growth of crops at 37°C, frequent cell recultures at the beginning are necessary, to fully restore the normal growth rate of the crop. Infectious environmental factors, the most common of which are fungi and bacteria, are often an obstacle to maintaining an in vitro cell culture. For this reason, all cell cultures are handled within a nematically flow chamber in order to achieve sterile conditions for the cells. Sterilization of the cell area is achieved on a daily basis by exposure to ultraviolet radiation for at least 15 minutes, while ethanol solution (70% v/v) is used for local sterilization of the site. The materials used are sterilized in a special liquid sterilization furnace (automatically) at 120°C and pressure 1 kp/cm^2 for 20 minutes.

2.6 Investigation of cell vitality

3. Synthesis and characterization of organic hybrid microspheres

The purpose of this work on the synthesis of organic-inorganic microcontainers is to create a material where it can be used by industry for the ultimate purpose of self-healing of concrete. In other words, a study was carried out to find a synthetic path that is easy, fast, but also easily adaptable to the final product. For these reasons, polymer microspheres were combined, changing parameters in the experimental process to achieve a size of microspheres, approximately 1 μm. The purpose of using microspheres at this size was to interact with *St. aureus*, the size of which is 0.5–1.5 μm.

3.1 Water traps

The purpose of producing water traps with a desired size of 1 μm and then incorporating them into the material is when they come into contact with the bacterium and their nutrient, to adsorb and retain the bacterium on their surface. Spherical water traps were made up by a two-step process, which initially includes the cross-linked polymethacrylic acid (PMAA) spheres through submersion polymerization after distillation, and then the conversion of carboxylic groups into corresponding calcium salts through the treatment of microtraps with Ca $(OH)_2$ solution. Methacrylic acid (MAA) and dimethacrylate glycolic acid ester (EGDMA) were used as monomers in a crusader role, in acetonitrile solvent with azodisisobutyronitrile (AIBN) beginning. Acetonitrile, being a nonprimary, polar solvent, favors polymer-polymer interactions, that is, hydrogen bonds between the carboxylic edges of polymer chains. MAA together with EGDMA are dissolved in acetonitrile in a 250 ml triple-blooded spherical flask. A freezer, thermometer, and N_2 supply are installed in this bottle. After an hour of stirring, the AIBN is added, which has been dissolved in a solvent quantity before. Stirring continues at the same temperature for another hour. In order to distill the solvent, the temperature of the experiment increases to the boiling point of acetonitrile (80°C). After a certain amount of solvent has been collected, the reaction is terminated, and the resulting final solution is in the form of an emulsion. Finally, after the solution is allowed to reach ambient temperature, the sample is centrifuged twice, rinsed with acetonitrile, and then dried. PMAA spheres acquire the ability to absorb water when the carboxylic groups are converted into the corresponding calcium salts. The experimental procedure is as follows: 2 g PMAA spheres dissolve in acetonitrile and spread through ultrasound. After the sample has been dispersed homogenously, add 0.74 g of 0>1 M Ca $(OH)_2$ solution. The mixture becomes clear (depending on the ratio of monomers—the less EGDMA is added, the clearer the solution becomes). After stirring for 30 minutes, the sample is centrifuged, rinsed with acetonitrile, and left to dry. Four different sized water traps were made up, changing the ratio of monomers in each case (**Figure 2**).

One can conclude from **Table 1** that the submersion polymerization after distillation gives uniform polymeric microspheres with different functional groups. **Figure 3** shows the SEM image of sample 2 together with the elemental analysis 2 before **Figure 3A** and after **Figure 3B** treatment with Ca $(OH)_2$.

According to the SEM images and the data in **Table 1**, the experimental process of composing water traps shows repeatability by giving spherical particles, with a similar diameter and small size distribution. The diameter shown in **Table 1** was calculated from the average of the diameters of hundreds of water traps with the corresponding standard deviation of each. The dispersion indicator is close to one and proves that there is a good size distribution. Also, the elemental analysis

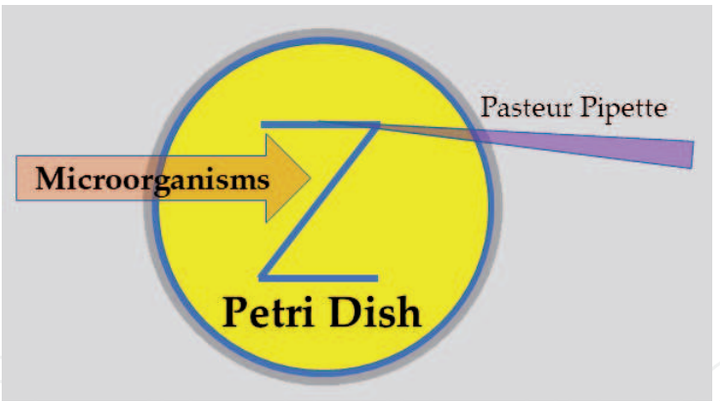

Figure 2.
Vaccination of microorganisms in petri dish.

Samples	n = [EGDMA]/[MAA] Molar ratio	Size (nm) (determined by TEM)
1	0.05	1004 ± 55
2	0.056	1150 ± 72
3	0.06	830 ± 40
4	0.085	626 ± 58

Table 1.
n = [EGDMA]/[MAA] molar ratio and size of water traps for 4 n compositions.

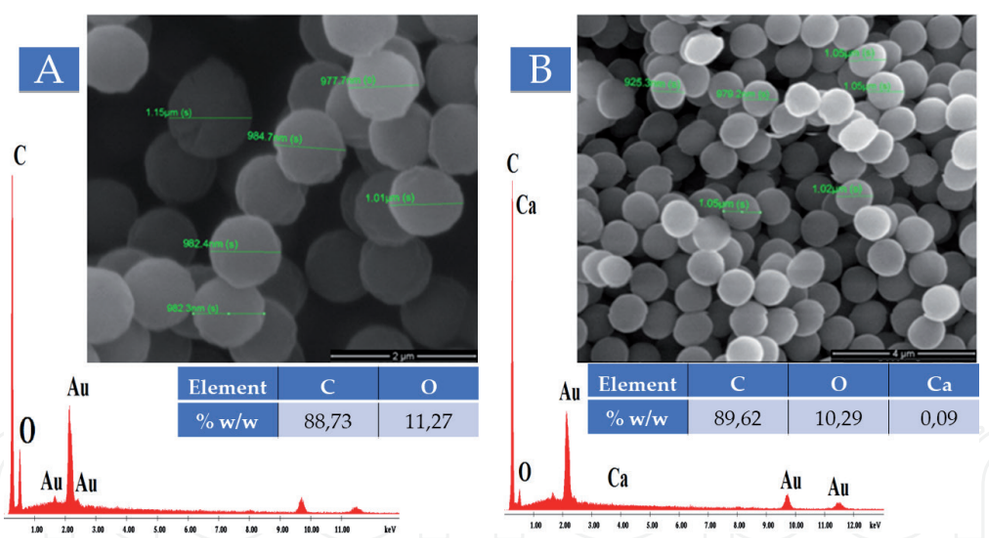

Figure 3.
(A) SEM image and EDS analysis of sample 2 as well as %w/w of data before modification with Ca (OH)2. (B) SEM and EDS analysis of sample 4 as well as the % w/w of data after modification with Ca (OH)₂.

(**Figure 3B**) in each sample case confirms the existence of Ca, after its modification, which means that the water microtraps are now capable of absorbing water. **Figure 3** confirms that statement via dynamic light scattering measurements.

For small amounts of water, up to 10% v/v of the total amount of solvent, the differences in hydrodynamic diameter are not significant, but as the percentage increases, there is a fairly large difference in size. This difference is evident when the percentage of water exceeds 15%, and this is probably due to the interaction of trap-water with water. These interactions may relate to the development of hydrogen bonds between particles and water but also between the particles themselves. The elucidation of the water absorbing mechanism needs further work (**Figure 4**).

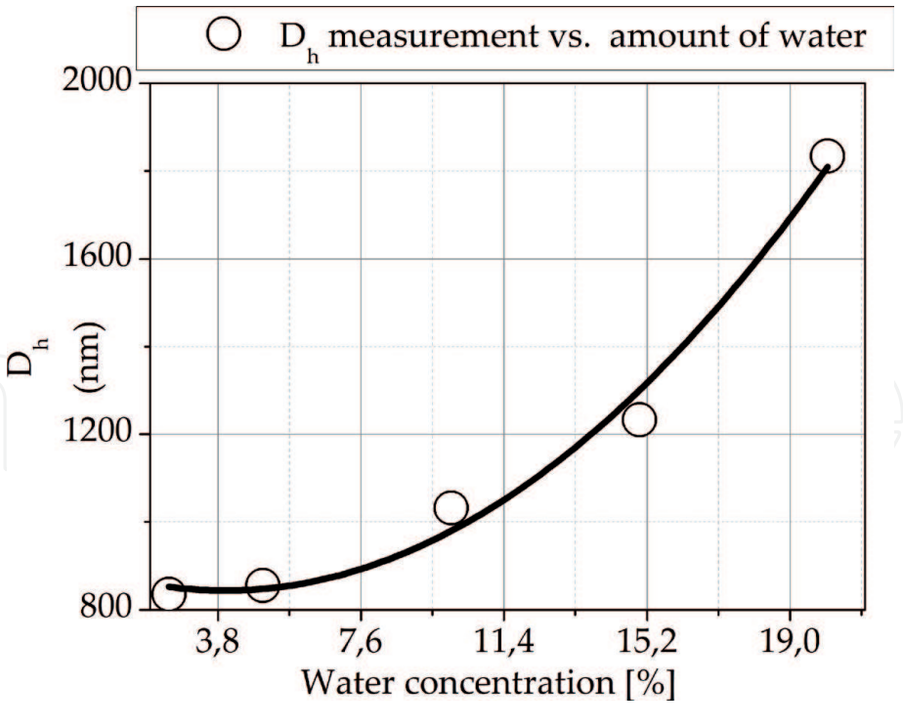

Figure 4.
Change in hydrodynamic diameter depending on different quantities of water.

3.2 $Ca(OH)_2$ @SiO_2 modified water traps

The purpose of the Ca $(OH)_2$@SiO_2 modification of the water traps is to give the adsorbed water traps a durable shell that will protect the bacterium and LB when it is introduced into cement. The SiO_2 shell synthesis process was done using the sol-gel method. For the more durable and complete coating of activated water traps, six experiments were carried out, each increasing the amount of the TEOS reagent. The experimental procedure is the same in all six experiments carried out and is as follows: in 150 ml boiling glass, the amount of traps is dissolved in acetonitrile, and the solution is left to ultrasound for 20 minutes and then for another while stirring, in order to spread the sample well in the solvent. Then the ammonia is added, and the stirring continues for another 15 minutes. TEOS is then added to the sample and the solution is left stirring for about 20 hours. Finally, each sample is centrifuged, rinsed, and left to dry. The whole experimental process takes place at ambient temperature. To make up the SiO_2 shell around the water traps, a water emulsion in oil (W/O emulsion) was created. Due to the organic solvent used (acetonitrile), when aqueous solution is added to it, water drops are created inside the solvent. In this case, due to the ability of water traps to retain water, the role of the aqueous phase in a W/O emulsion is made up of particles that have adsorbed water. The water comes from the solution of NH_3 30%. Thus, in the middle of the water traps and acetonitrile, when the precursor TEOS compound is added, hydrolysis and condensation take place there. This overlays silicon-shelled water traps (**Figure 5**).

Figure 6 shows the size of the nanospheres as a function of R_w (H_2O/ Si ratio). This graph was determined by keeping all parameters constant except the quantity of the added water in the experiments. Note that the amount of modified water microtraps covered was the same in all experiments, (0.5 g).

Figure 7 shows the SEM micrographs of the modified nanotraps. The SEM image and the corresponding EDS elemental analysis are reported for the sample of the experiment corresponding to the one with the largest amount of TEOS added.

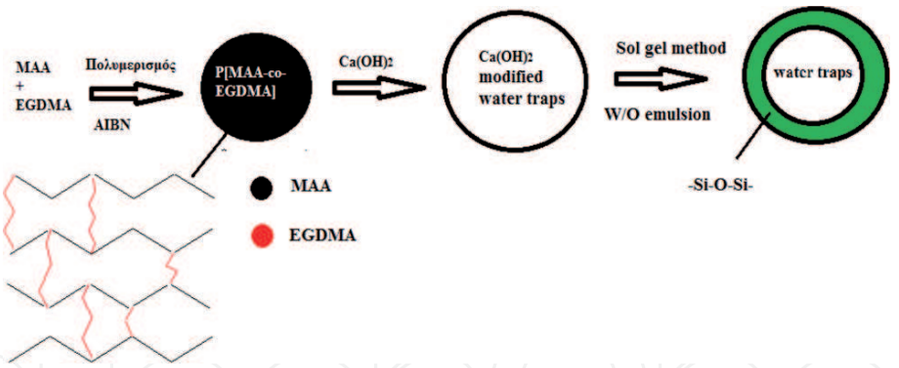

Figure 5.
Schematic representation of the coating of modified water traps with SiO_2 in a W/O emulsion.

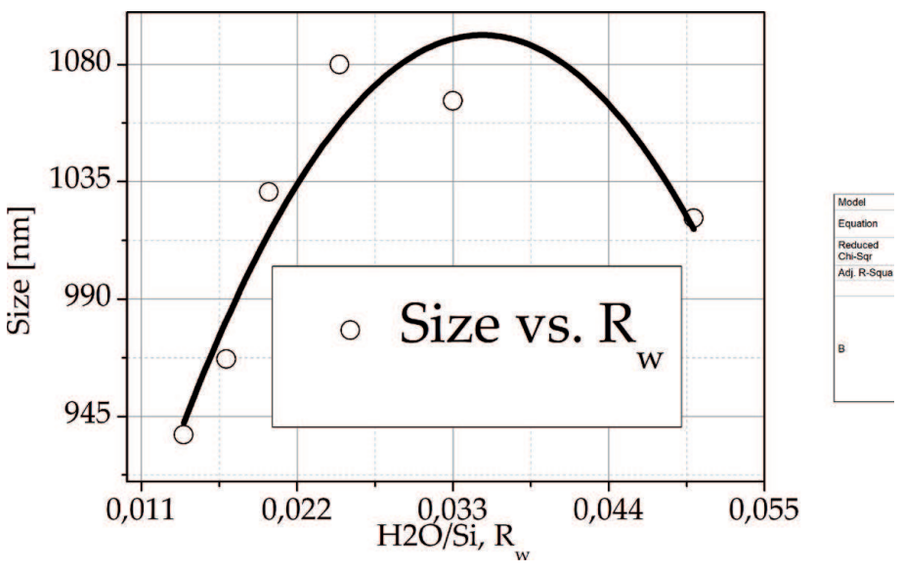

Figure 6.
The corresponding Rw ratio for each case of SiO_2@Ca $(OH)_2$ modified water traps.

Element	C	O	Si
% w/w	75,86	22,13	2,01

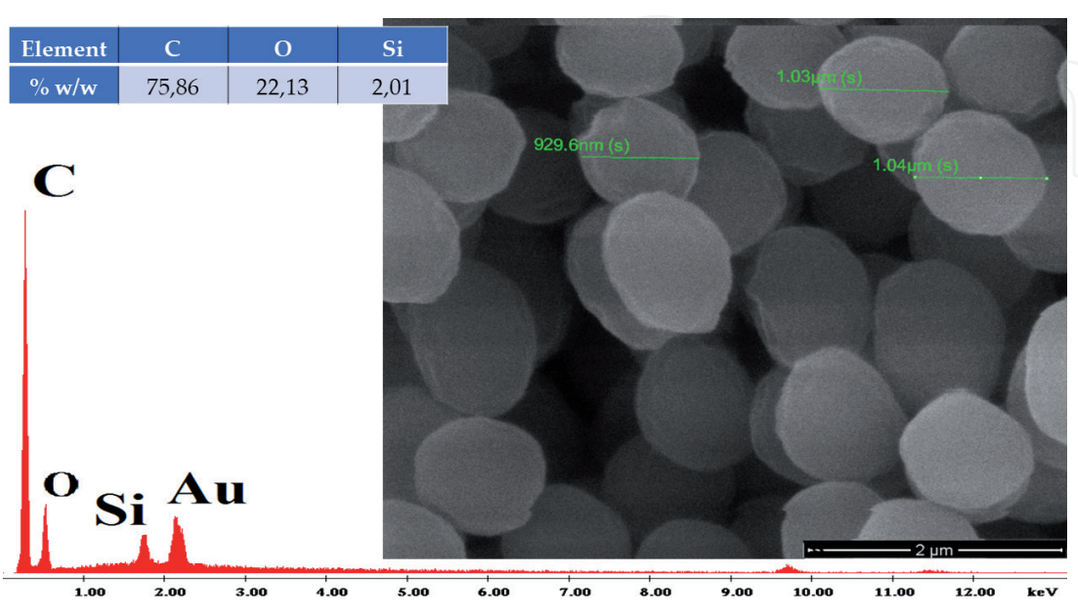

Figure 7.
SEM micrograph. EDS analysis of the experiment with 21 ml of TEOS as well as the %w/w of each element.

3.3 SiO_2 microspheres

The purpose of the study is to learn the behavior of bacteria with an inorganic microsphere of appropriate size. As mentioned above, silica is well compatible with cement and is therefore used as a small transport system. The synthesis process is relatively cheap and easily manageable. The desired ball size is about 1 μm. Through the sol-gel method, SiO_2 micro-containers were combined into two series of experiments by changing the parameters of the experiment while keeping the remaining constant. In the 1st series of experiments, we increased the amount of TEOS added. In the second series of experiments, the volume of the solvent increased. In both cases, the ratio of H_2O/Si, R_w and the change in size of the microspheres was investigated. We used ethanol, water, TEOS, and 30% NH_3 as the catalyst. The experimental SiO_2 ball synthesis process was done using 150 ml boiling glass in which EtOH and H_2O were mixed and left to stir for about 10 minutes. After that, we added NH_3 and stirred for 20 minutes. The reaction starts when the TEOS drip is added. Leave the final solution for about 20 hours under the same conditions, then centrifuge and dry. The whole experimental process takes place at ambient temperature. **Table 2** gives the ratios H_2O/Si, Rw for each case. This ratio results from the added water in each experiment. The water comes from the 30% NH_3 solution. That is, 0.3 ml H_2O is contained in 1 ml of NH_3. **Table 3** gives the exact amounts of chemicals used. The resulting SEM micrographs are shown in **Figure 8**. According to the SEM image, the SiO_2 microsphere synthesis process shows repeatability by giving spherical particles, with a similar diameter and small size distribution. The dispersion proves that there is a good size distribution.

The characterization of the size of SiO_2 microspheres was done with DLS. The study was carried out to find the optimal sample concentration for the correct measurement in DLS. In this case, it was found that the optimal concentration is 10 mg/L. According to the literature [3], a suspension is considered stable (i.e. not cracked) when it has a potential positive of +30 mV and more negative than -30 mV. According to the DLS measurement, the size of the SiO_2 microspheres is 782 ± 46 nm, which is also in line with the measurement of SEM. The ζ-potential is −56 ± 0.361, which attests to the stability of the sample composed. In summary, the synthesis of an inorganic microspheres of about 1 μm size was achieved by the sol-gel.

Experiment	TEOS (ml)	[H_2O/Si] (R_w)
1	4	3.2
2	8	1.6
3	10	1.28
4	12	1.06

Table 2.
The ratio R_w resulting by changing the amount of TEOS in the first case of experiments, keeping the remaining parameters constant.

Experiment	EtOH (ml)	H_2O (ml)	[H_2O/Si] (R_w)
1	100	11	1.28
2	200	22	2.38

Table 3.
The ratio Rw, which results by changing the amount of solvents in the second case of experiments, keeping the remaining parameters constant.

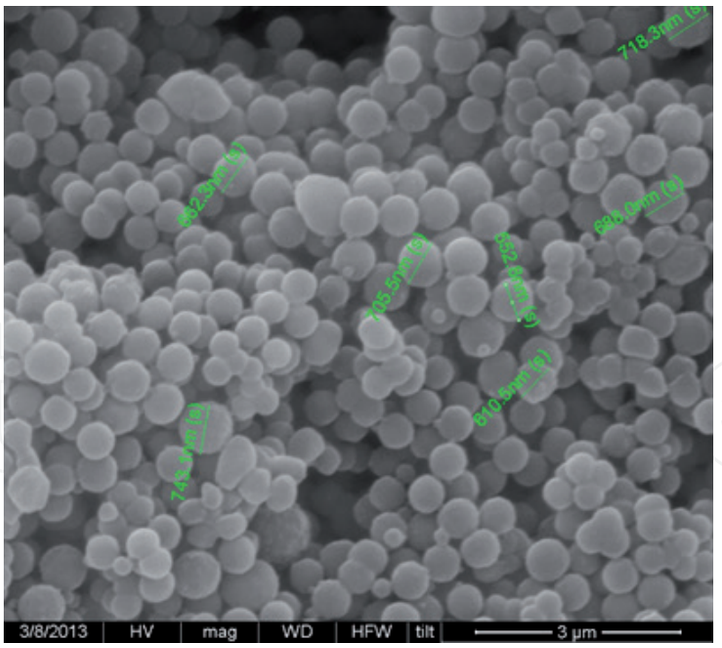

Figure 8.
SEM image of 725 ± 84 nm size SiO2 microspheres.

3.4 Polymethyl methacrylate microspheres

The purpose of the study is to verify the behavior of bacteria with an organic microcontainers of appropriate size. The method is economical and easily manageable. Desired sphere size is about 1 μm. Polymeric methyl polymethacrylate microspheres (PMMA) were made up with radical polymerization and in particular with emulsion polymerization. The reagents we used are methyl methacrylate monomer (MMA), KPS start-up and water solvent. As the desired sphere size is approximately 1 μm, experiments were carried out changing the following parameters of the experiment: (a) the amount of solvent, (b) the amount of the authority, and (c) the amount of water. The experimental procedure was carried out in a 100 ml spherical flask, the solvent was added where, with the help of a heating plate under stirring, the temperature was maintained at 70°C. Then the monomer was added, and after 20 min stirring, the starter was added. The colloidal solution was left stirring for approximately 20 h. Polymerization took place under a nitrogen atmosphere. The following describes the puma synthesis mechanism consisting of (1) the starting stage where the authority gives free radicals and reacts with the monomer, (2) the phase of propagation is observed polymer development, and (3) the stage of termination at which two roots react with each other, and the polymerization is terminated by taking PMMA as a product. **Figure 9** summarizes the procedure (**Table 4**).

Table 5 gives the quantities of reagents that led to PMMA microsphere synthesis with the largest size, in each of a series of experiments.

Figure 10 shows the size of the microcontainers as a function of water (A), MMA (B), and C starter.

According to the above diagrams, it is observed that the largest size of PMMA microbeads synthesized results from the second series of experiments, i.e., by changing the amount of monomer by keeping the remaining parameters of the experiment constant. **Figure 11** shows the micrograph of the samples by SEM.

The SEM image above shows the size of the PMMA micro-containers with the largest diameter achieved. As observed from the above image, the sample shows a large multi-dispersion. The characterization of the size of PMMA micro-containers was subsequently made with the dynamic scattering of light. As described in the

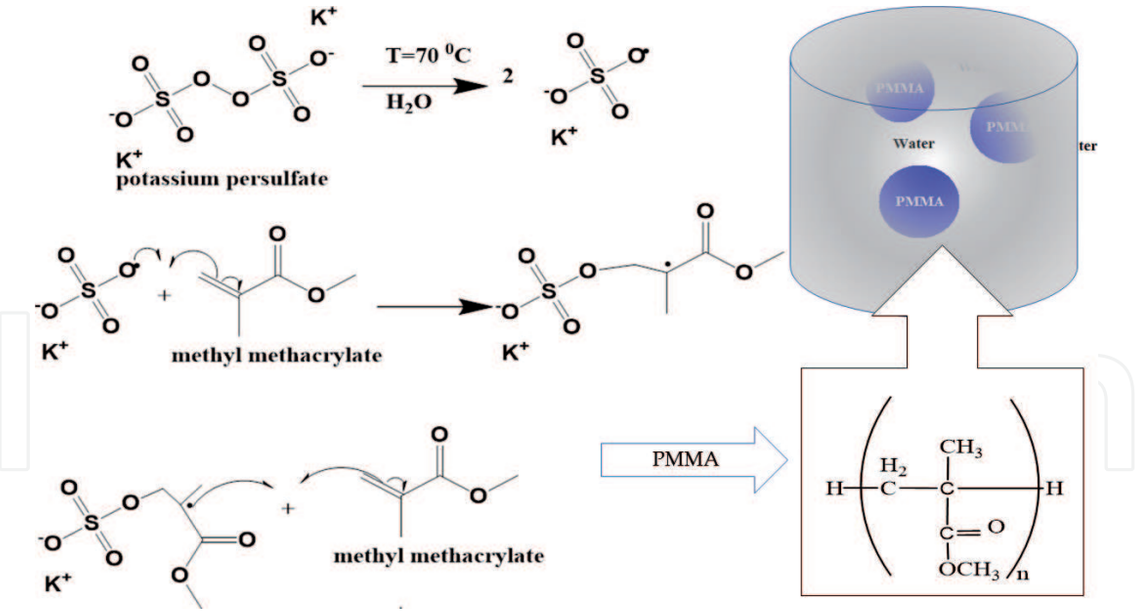

Figure 9.
PMMA microcontainer synthesis mechanism.

	Size (nm) Determined by SEM
1η series of experiments	725 ± 84
2η series of experiments	587 ± 38

Table 4.
The size of microspheres resulting from both series of experiments.

Change	MMA (ml)	KPS (mg)	Water (ml)	Size (nm, TEM)
Solvent	4	40	100	417 ± 23
Monomer	6	40	30	711 ± 90
Starter	4	40	30	299 ± 26

Table 5.
Quantities of reagents in each series of experiments with the corresponding size resulting each time.

case of SiO_2 micro-containers, a study was carried out to find the optimal sample concentration for the appropriate measurement of DLS. In this case, it was found that the optimal concentration is 10 mg/L. The following diagram, in addition to the size of the micro-containers resulting from the measurement, also lists the z-potential of the sample. This studies whether or not the suspension is stable. According to the DLS measurement, the resulting PMMA size is 780 ± 25 nm, which is in line with the SEM measurement. The ζ-potential is −38.6 ± 0.651, which attests to the stability of the samples. PMMA microsphere synthesis is economical, easily manageable and takes place in aqueous environment. For the production of a product, in industry, the above factors are very important.

3.5 Microcontainers with polyurea shell

The ultimate purpose of this study is to trap the bacteria spores in the microcontainers with polyurea shells. This can be done if the spores of the microorganism are scattered in the drops of the oily phase, and the shell forms around them.

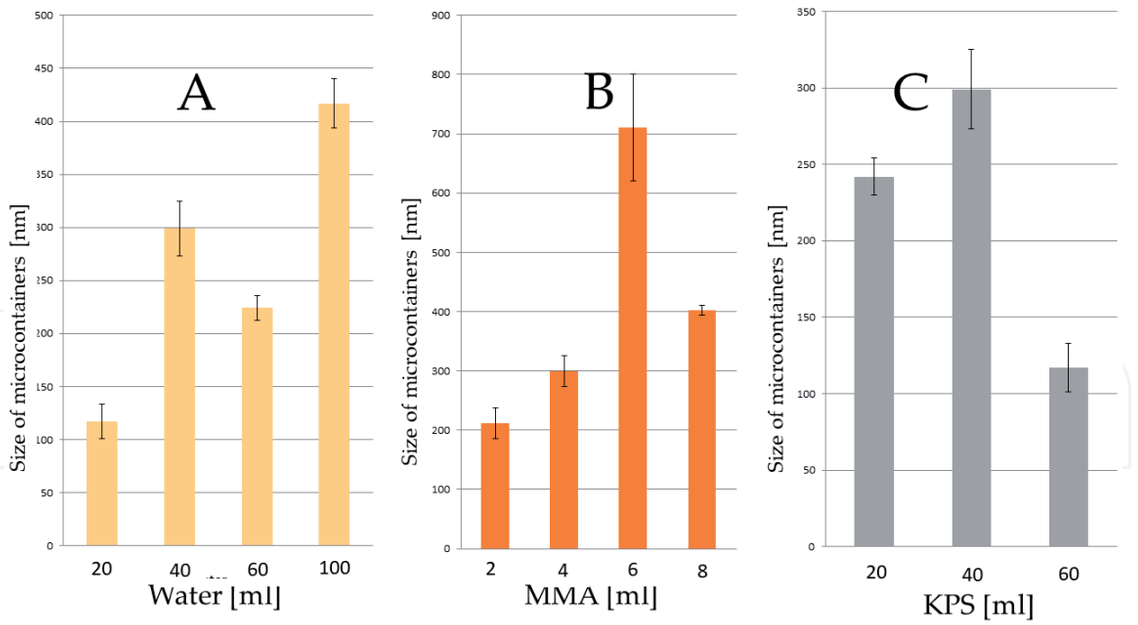

Figure 10.
(A) PMMA microsphere size changing the amount of solvent, (B) PMMA microsphere size changing the amount of monomer, and (C) PMMA microsphere size changing the amount of the beginning.

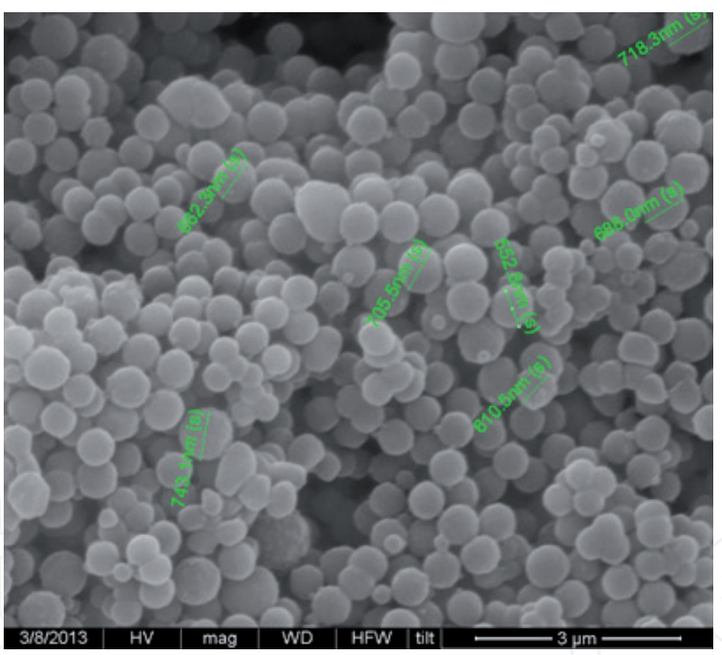

Figure 11.
SEM image of the PMMA micro-container with a size of 711 ± 90 nm.

The synthesis of polyurethane-coated microcontainers is achieved through interfacial polymerization (IP) in an oil emulsion in water (O/W emulsion). The synthesis of polyurea shell micro-containers is achieved by polymerization on the middle surface of two liquids that are not mixed together. This study investigated an oil emulsion in water (O/W emulsion). The reagents used are organic soluble monomer (toluene diisocyanate [TDI]), water-soluble monomer (ethylenediamine, EDA, diethylenediamine, DETA), ethylenediamine (EDA), oil phase (1-octadecene, paraffin), water phase (H_2O), emulsifier (sodium sulphonate dodecyl, SDS), methyl ester poly ethylene glycol (PEG), polyvinylpyrrolidone (PVP), Triton x-100, catalyst (NH_4Cl) and solvent (acetone). Four series of experiments were carried out by changing some parameters, keeping the remaining constants, in order to synthesize

polyuria shell containers. The experimental procedure followed in all four series of experiments is as follows: For the composition of the oily solution in a boiling glass, mix the organic soluble monomer with the organic phase and acetone. The stirring lasts a few minutes. The oil emulsion in water is formed when the oil solution is added to 50 ml of aqueous solution containing 5% w/w emulsifier. Stirring of the system takes place at ambient temperature and at 300 rpm/min for 5 min. The water-soluble monomer solution in the emulsion is added drip and stirring at 600 rpm. Then add 5% w/w NH_4Cl and stir continuously for 3 h at 60°C. Finally, the sediment is centrifuged, washed with 30% ETOH, and left for drying [4]. In the first series of experiments, the possibility of forming polyurea shell micro-containers using two different water-soluble monomers (EDA and DETA) with an emulsifier SDS is studied. All other factors in the experiment remain the same. In the second series of experiments, the possibility of different emulsifiers (SDS, PEG, PVP, and Triton x-100) for the formation of polyuria shell micro pots is studied, using DETA as a water-soluble monomer. All other factors in the experiment remain the same. The third series of experiments studies the action of different phases of oil (1-Decaoctene and paraffin) using Triton x-100 as an emulsifier and as a water-soluble monomer DETA. All other factors in the experiment remain the same. The fourth series of experiments studies the dependence of the composition of micro pots on the quantity of peg emulsifier. Paraffin was used as an oily phase and DETA was used as a water-soluble monomer. All other factors in the experiment remain the same. **Figure 12** shows the synthesis of micro-containers of polyurea with different amines.

The mixture of the oil phase, containing the oil and TDI, is dispersed in the aqueous phase with the emulsifier, thus forming an oil emulsion in/water (O/W emulsion). Initially, in the aqueous phase, the hydrophobic groups of the emulsifier are covered by its hydrophilic groups, in such a way that micelles are formed (**Figure 10**). Adding the oil solution, the hydrophilic groups are diffused into

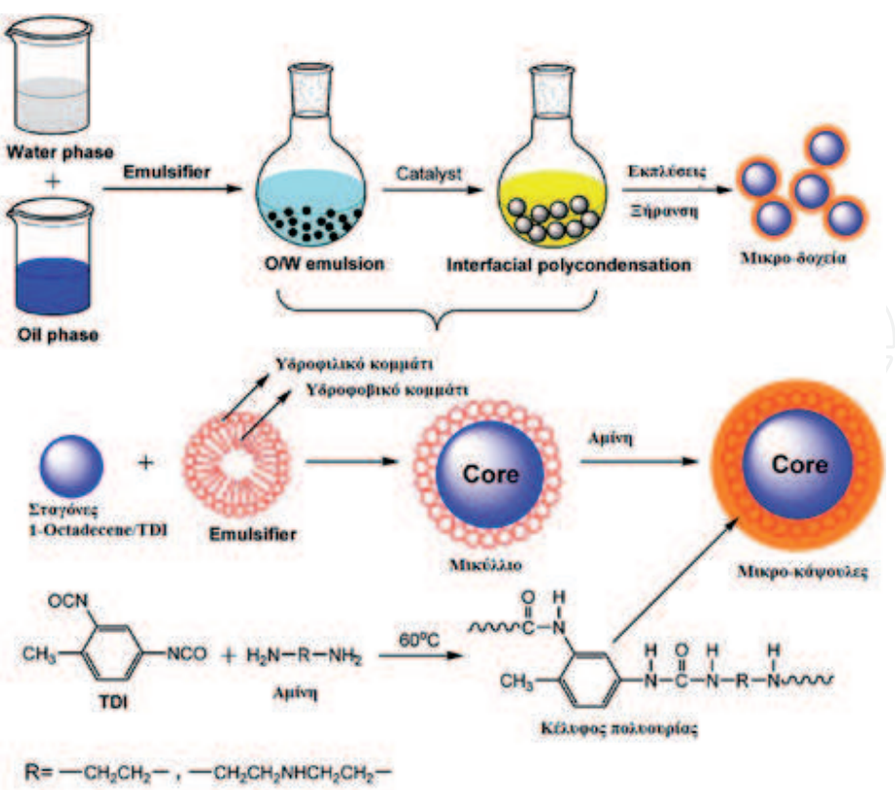

Figure 12.
Representation of the composition of polyurea micro-containers with different amines.

the oil, while the hydrophilic along the drop covering it. The formation of the shell begins when regional isocyanate groups of TDI are hydrolyzed in the oil-water middle surface forming amines, according to the reaction:

$$RNCO + H_2O \rightarrow RNH_2 + CO_2 \quad (1)$$

These amines react with non-hydrolyzed isocyanate groups, thereby forming a polyuria network. When the original shell is formed, the added amine (the water-soluble monomer) must penetrate the membrane and penetrate the oil phase to react with TDI (the organ soluble monomer). This makes the polyurea shell denser and more durable. As a result, the formation of a polymer shell (consisting of a polyuria network) is observed on the interface of the emulsifier and the oil phase, due to the reaction between amine and TDI. The temperature that the reaction takes place is the ambient temperature [4]. The series of experiments on the composition of polyuria shell micro-containers were carried out in order to find the most suitable experimental conditions for an efficient result. Thus, the experiments are related to each other by following each time the most promising result. Thus, in the first series SDS is used as an emulsifier and EDA and DETA are used as amines. Between the two amines, the other studies used DETA, as it showed the best micro-pot formation. The second study showed that PEG and Triton x lead to the formation of micro-containers. Below is the SEM image of the sample where DETA has been used as an amine while PEG has been used as an emulsifier. **Figure 13** shows SEM of the sample of polyurea shell containers from the second series of experiments, emulsifier PEG.

As well, the study of specific microcontainers is indicative that an average microsphere size has not been found as in all previous microsphere studies. The above image is not representative for the entire sample. An indicative study of the composition of microspheres with polyuria shell was carried out in this section. Polyuria has very good strength and is insoluble in most solvents. That is why it was chosen to study such a system. Experiments were conducted changing parameters of the experiment. Promising results give those cases where PEG and Triton x with amine DETA are used as emulsifiers and as an oil phase 1-octadecene.

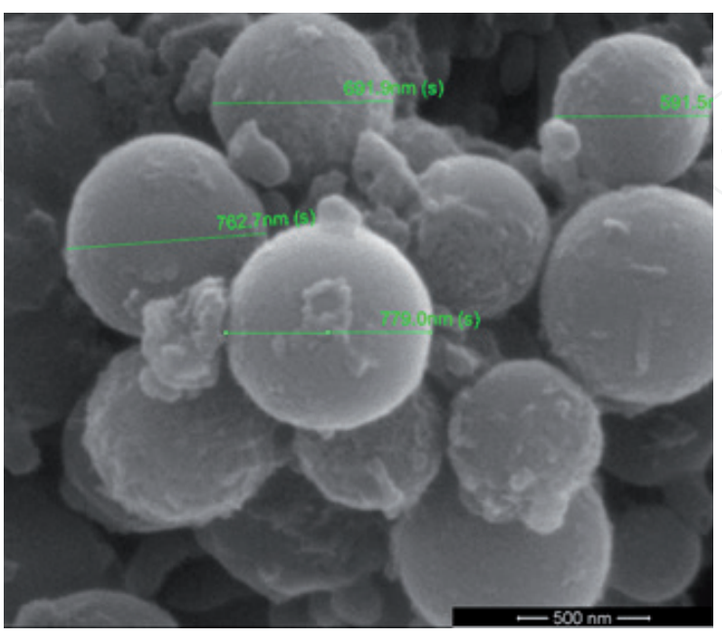

Figure 13.
SEM image of the sample of polyurea shell micro-containers from the second series of experiments, emulsifier PEG.

3.6 Microbial precipitation $CaCO_3$

As has already been mentioned, the application of bacteria in concrete for the purpose of self-healing in case of cracks is not impossible. There have been many studies that prove their effectiveness in healing cracks. Bacteria that can withstand the extreme environment of concrete (high voltages and intense alkalinity) are those of the genus Bacillus (Gram-positive bacteria). Species of this genus have the potential to form endospores, forms resistant to non-bacterium-friendly environments. When the endospores are found in appropriate conditions, they are activated (a process also known as eblastosis) and begin to develop. Bio-precipitation of a mineral by a microorganism occurs when it is found in an environment with suitable nutrient material. In the case of cement self-healing, the mineral chosen is $CaCO_3$. Although the most suitable bacterium for this work is some species from the genus Bacillus, this study looked at two other types of bacteria, namely *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). Thus, the possibility of submersion of $CaCO_3$ salt from a Gram-negative and a Gram-positive bacterium was studied, with the main emphasis on *Staphylococcus aureus*. The reason this was done is because *St. aureus* has a cell structure similar to that of Bacillus, although it does not have the ability to form endospores. It should be stressed that the bacteria studied in this work were pathogenic, and all safety rules were followed during their use. As mentioned in the above section, $CaCO_3$ precipitation is a two-way chemical process controlled mainly by four factors, first calcium concentration, second concentration of dissolved inorganic carbonate ions (DIC), third the pH, and fourth availability of nucleation centers.

There are three mechanisms associated with bio-precipitation, and in this work, the mechanism of urea breakdown (hydrolysis of urea, HU) was studied through the enzyme urease, a course easily manageable and controlled. The general reaction is as follows:

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \tag{2}$$

The bacterium plays the role of the nuclearization center with the following mechanism:

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \tag{3}$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \downarrow \tag{4}$$

The exponential growth phase is the ideal phase to study any cellular function.

3.6.1 Precipitation $CaCO_3$ from bacteria

3.6.1.1 $CaCO_3$ precipitation rating study by Staphylococcus aureus

In order to investigate the possibility of precipitation of $CaCO_3$ from *St. Aureus*, four microorganism solutions with a different amount of $CaCl_2.2H_2O$ and urea were prepared. In this way, the ability of this bacterium to precipitate salt at different nutrient concentrations has been qualitatively studied. **Table 6** gives the composition of *St. aureus* solutions with different concentration of $CaCl_2.2H_2O$ and urea.

For the preparation of the above solutions, a quantity of microorganism solution was isolated and the corresponding quantity of $CaCl_2.2H_2O$ and urea was added

Materials	1° Solution	2° Solution	3° Solution	4° Solution	5° Solution
$CaCl_2.2H_2O$	—	0.05 g	0.1 g	0.25 g	0.5 g
Urea	—	0.05 g	0.1 g	0.25 g	0.5 g
Cultivation St. aureus	20 ml	20 ml	20 ml	20 ml	20 ml

Table 6.
Composition of St. aureus solutions with different concentrations of $CaCl_2.2H_2O$ and urea.

each time. The solutions were incubated at 37°C at 100 rpm. After 72 h, in these conditions, the solutions were removed and compared. The solution of the microorganism containing the most $CaCl_2.2H_2O$ and urea showed the greatest turbidity of the other solutions with apparent precipitation of $CaCO_3$. There is a marked change in the turbidity of the solutions, as distinguished from the images above. The change in clarity between the nutrient solution, LB and the solution after the development of the microorganism, certifies that the microorganism has been properly incubated to study any of its metabolic processes. An apparent precipitation of sediment is observed between the 5th solution and the microorganism solution (**Figure 14C**). *St. aureus* solution becomes cloudier while $CaCO_3$ submersion certifies its ability to break down urea and lead to precipitation of the mineral. In order to characterize more fully the precipitation of $CaCO_3$, a kinetic study is carried out by the microorganism in solutions of (a) in nutrient material LB in the presence of $CaCl_2.2H_2O$ and urea and (b) in agar medium (Petri dish) in the presence of $CaCl_2.2H_2O$ and urea. Taking samples at regular intervals, and through infrared spectroscopy (FT-IR), the existence of the mineral is studied in two different environments. The following describes the composition of microorganism solutions in LB nutrient solution and

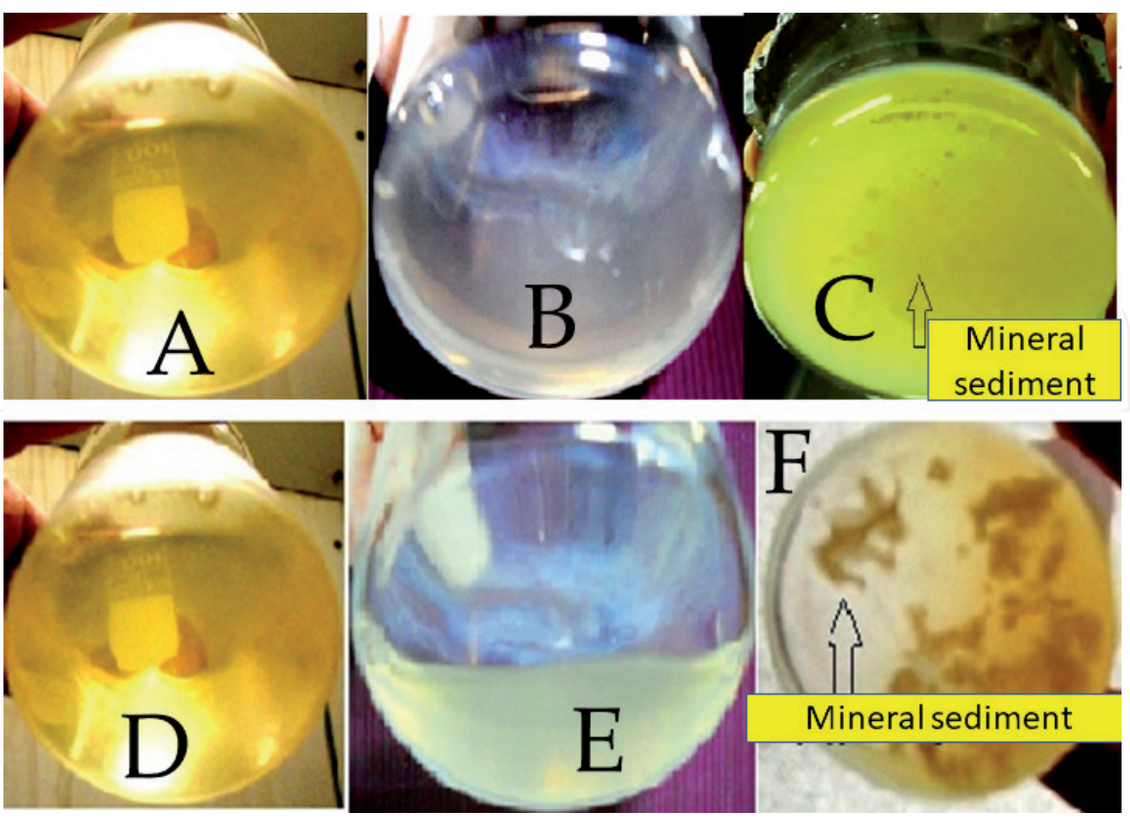

Figure 14.
Change in clarity of St. aureus solution. (A) LB solution, (B) microorganism solution after incubation in LB, and (C) precipitation of $CaCO_3$ from the 5th solution of the bacterium. Change in the turbidity of the E-coli crop: (D) LB solution, (E) microorganism solution after incubation in LB, and (F) precipitation of $CaCO_3$.

agar nutrient medium for the $CaCO_3$ precipitation kinetic study. Maximum concentrations of $CaCl_2.2H_2O$ and urea were selected from $CaCO_3$ precipitation quality control, as in these quantities the mineral was more evident.

In this particular case, however, *St. Aureus*, which develops, is placed in the stirring incubator for approximately 24 days in certain conditions (37 °C, 100 rpm). At regular intervals, a sample is isolated, centrifuged, and sterilized to measure the FT-IR spectrum for the purpose of certifying the presence of $CaCO_3$.

In this particular case, however, o *St. aureus* is left in the incubation oven for about 21 days under certain conditions. (37 °C). At regular intervals a sample is isolated, centrifuged, sterilized and measured the FT-IR spectrum for the purpose of certifying the presence of $CaCO_3$. The environment in which the mineral develops is quite complex, with many different compounds, which come from the nutrient and microorganism. These compounds show peaks in IR spectra. Therefore, in order to verify the existence of $CaCO_3$ in the cases where the study is carried out, a comparison of the range of $CaCO_3$ with the spectrum $CaCO_3$ LB nutrient solution (**Figure 15A**), in order to identify the tops of the mineral in the nutrient microorganism. Then, after determining the carbonic ion peaks in the IR spectrum, each kinetic study spectrum is included in addition to the spectra of the isolated samples, the $CaCO_3$ LB and the spectrum of the microorganism. In this way, it is possible to identify the peaks corresponding to the $CaCO_3$ in any case but also the peaks corresponding to the microorganism. The results of this study are listed below.

Since $CaCO_3$ is a crystalline salt, the vibrations that occur in an IR spectrum correspond to the bonds of CO_3^{2-}. The carbonic ion peaks in the IR spectrum are as follows: a strong wide peak in the 1530–1320 cm^{-1} range, medium intensity peaks at 1160 cm^{-1} and in the 890–800 cm^{-1} range, and a peak in the 745–670 cm^{-1} range [5]. Based on the above and the IR spectrum of **Figure 15A**, we observe that $CaCO_3$ in LB solution shows peaks at 712, 873, and 1409 cm^{-1}. The top at 1638 cm^{-1} can be attributed to any of the components of the nutrient medium LB. Finally, the peak shown in the range 3000–3500 cm^{-1} is attributed to the –OH of the aqueous solution. An indicative range of IR bacterium is shown in **Figure 15B**. Based on

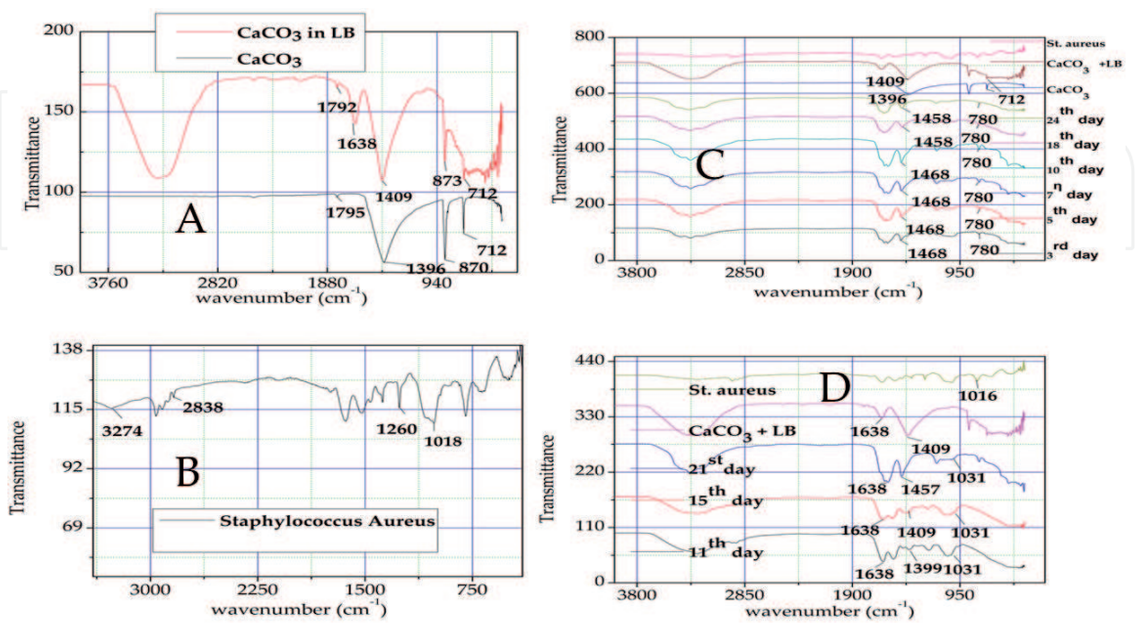

Figure 15.
(A) IR spectrum of the crystalline $CaCO_3$ and $CaCO_3$ in the nutrient medium LB, (B) spectrum IR St. aureus in nutrient medium LB, (C) IR spectrum of $CaCO_3$ precipitation kinetic study from St. aureus to nutrient LB, and (D) IR spectrum of $CaCO_3$ kinetic precipitation study from St. aureus in the nutrient agar medium in a dish petri.

the range of the bacterium, we can assign some peaks to the various components that make up the microorganism. The peak at 1018 cm^{-1} is attributed to polysaccharides compounds, the peak at 1260 cm^{-1} at the asymmetric vibration of the PO_2 cm^{-1} bond, at 3274 cm^{-1} we observe the vibration of N-H, while at 2838 cm^{-1} we observe the symmetrical vibration of the CH_2 bond [5]. The IR spectrum of the $CaCO_3$ kinetic precipitation study from *St. aureus* to the nutrient medium LB is given below. By comparing the spectra of the samples isolated each time, the spectra of the microorganism and $CaCO_3$ in LB draw the following conclusions. From the 3rd day of incubation of the microorganism with $CaCl_2.2H_2O$ and urea, precipitation $CaCO_3$ is observed. This is certified from the top at 1468 cm^{-1}, which as mentioned above belongs to the display area 1530–1320 cm^{-1} for carbonate ions. The new peak at 780 cm^{-1} is also attributed to carbonate ions. Although this peak does not correspond to any area of appearance of carbonate ions, it can be attributed to the range 745–670 cm^{-1} but as shifted. The presence of carbonate ions is observed in the kinetic study for all periods of time when the sample is isolated. The remaining peaks showing the sample spectra can be attributed to compounds of the microorganism and nutrient material. Finally, with this comparative study of the various IR spectroscopy, it appears that *St. aureus* is capable of precipitating $CaCO_3$ in LB nutrient solution when dissolved in sufficient $CaCl_2.2H_2O$ and urea. Due to the ureolytic action of the microorganism, carbonate ions are released, and in a calcium-rich environment there is precipitation of the mineral. Below is the IR spectrum of the kinetic precipitation study $CaCO_3$ from *St. aureus* to the nutrient in Petri dish. The comparative precipitation study of $CaCO_3$ by St. aureus in the nutrient medium, in a Petri dish, observed the carbonic peaks corresponding to carbonate ions in the isolated samples. Thus, according to the above range, the IR peak at 1638 cm^{-1} is attributed to nutrients and the peak to 1031 cm^{-1} to the polysaccharides of the microorganism. The peak attributed to carbonate ions is in the range 1399–1457 cm^{-1}, as it is included in the range 1530–1320 cm^{-1} where these ions appear. We can conclude, for example, that *St. aureus* has the ability to precipitate $CaCO_3$ in a Petri dish (**Tables 7** and **8**).

3.6.1.2 Precipitation characterization study of $CaCO_3$ in microorganism solution

In this way, the ability of this bacterium to precipitate salt has been qualitatively studied. **Table 9** shows the composition of E-coli solutions with different concentrations of $CaCl_2.2H_2O$ and urea.

For the preparation of the above solutions, a quantity of microorganism solution has been isolated, and the corresponding amount of $CaCl_2$ and urea every time. These solutions were incubated at 37°C and 100 rpm. The solution of the microorganism that contained the most amount of $CaCl_2.2H_2O$ and urea showed the greatest turbidity of the other solutions with apparent precipitation of $CaCO_3$. After 72 h in these conditions, the solutions were removed and compared. Below are

Materials	Quantities
LB	49.5 ml
$CaCl_2.2H_2O$	2.5 g
Urea	2.5 g
Solution St. aureus	0.5 ml

Table 7.
Quantities of materials used for the kinetic precipitation study $CaCO_3$ from microorganism solution to LB nutrient material.

Materials	Quantities
Nutrient agar medium	25 ml in a dish Petri
$CaCl_2.2H_2O$	0.5 g
Ουρία	0.5 g
Solution St. aureus	Capable of its growth in the dish

Table 8.
Quantities of materials used for the kinetic precipitation study of $CaCO_3$ from microorganism solution to a nutrient agar medium in a petri dish.

Materials	1° Solution	2° Solution	3° Solution	4° Solution	5° Solution
$CaCl_2$	—	0.05 g	0.1 g	0.25 g	0.5 g
Urea	—	0.05 g	0.1 g	0.25 g	0.5 g
Cultivation *E-coli*	20 ml	20 ml	20 ml	20 ml	20 ml

Table 9.
Composition of E-coli solutions with different concentrations of $CaCl_2.2H_2O$ and urea.

the photos of the nutrient solution, the solution of the bacterium after incubation, and the 5th solution. As shown above, during the development of the microorganism *E-coli,* a comparative study is carried out, and there is a marked change in the turbidity of the solutions. The change in clarity between the nutrient solution, LB and the solution after the development of the microorganism, certifies that the microorganism has been properly incubated to study any of its metabolic processes. **Figure 13** C has a marked turbidity in the solution of the microorganism that contained the most $CaCl_2.2H_2O$ and urea, in relation to the incubation solution of the microorganism. The sediment submerged can be attributed to the creation of $CaCO_3$ from the *E-coli*. The visual characterization of calcium carbonate precipitation shows that the *E-coli* through the ureolytic breakdown sink $CaCO_3$. Two types of bacteria have been studied in this paper, *E-coli* and *St. aureus*, for their ability to break down urea and precipitate $CaCO_3$ in an environment rich in Ca^{2+}. Through the quality tests carried out, it was observed that both bacteria precipitate the desired mineral. Further characterization was carried out with the *St. aureus,* where a kinetic study was carried out on the existence of $CaCO_3$, in two different nutrient environments of the microorganism. In both cases, precipitation of the mineral. These bacteria in force can be used in the phenomenon of self-feeding of cement, since it is evident that they can precipitate $CaCO_3$ under certain circumstances.

3.7 Interaction between microbeads and microorganisms

In this chapter, bacteria behavior is studied in the presence of microspheres. Specifically, the interaction between microspheres and bacteria in vitro is studied. With the ultimate aim of these micro-containers being introduced into cement as carriers of bacteria, it is necessary to study whether polymer spheres are toxic to humans and bacteria. Of the five types of polymeric microspheres compositional (a) water traps, (b) coated water traps with SiO_2, (c) SiO_2 microspheres, and (d) PMMA microspheres for their interaction with bacteria were studied. The fifth type of polymeric micro-cell, polyuria shell micro-containers, needs further optimization before any coupling with a microorganism. Following this work, it is studied whether microns affect the ureolytic action of bacteria for $CaCO_3$ submersion. In other words, it is being studied whether the bacteria are still capable of submerging the mineral after contact with the microcontainers (**Figure 16**).

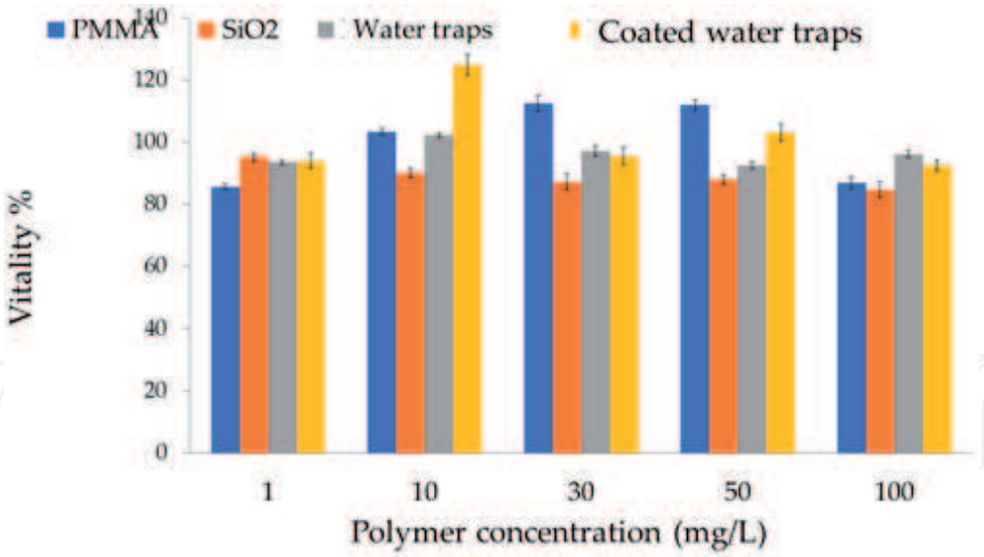

Figure 16.
HEK-293 cell vitality rates, presence of microspheres at various concentrations.

3.7.1 Toxicity study

3.7.1.1 Study of the cytotoxic action of organic-inorganic microspheres

The investigation of cell vitality is a characteristic indicator and a necessary condition in cases of cytotoxic activity of polymeric inorganic-organic microspheres where they will be used in materials that will come into direct contact with humans, such as cement. The MTT method studies the cytotoxic activity of the microspheres that were compositional in the HEK-293 cells. Studied, the cytotoxic action of microspheres at various concentrations. The rates of cell vitality after incubation with these materials are shown in the **Figure 14**. The diagram shows that the microspheres studied are not toxic to humans. In general, we conclude that regardless of polymer concentration the metabolic function of cells is not affected in the presence of micro-transfer systems.

3.7.1.2 Toxicity study of organic-inorganic microbeads to St. aureus

After it has been shown that microspheres are not toxic to humans, it was then investigated whether microspheres affect the development of the microorganism. In other words, quality control is carried out on the toxicity of micro-transport systems in *St. aureus*. Quality control is done by vaccinating the microorganism in Petri plates, in the presence of microspheres. In this case, however, before vaccinating the microorganism in the dish, four *St. aureus* solutions were prepared to which a quantity of each case of microspheres was added to each. The resulting sediment, consisting of microorganisms and microspheres, was inoculated in a Petri dish. Thus, the development of *St. aureus* was observed in an environment containing micro-transport systems. **Figure 17** shows photos of the Petri plates of *St. aureus* for each case of microcephaly studied.

According to **Figure 17**, it is certified that microspheres do not affect the development of *St. aureus*. The colonies formed by the microorganism are visible in all cases of plates. These colonies prove that *St. aureus* remains active metabolically even in the presence of microspheres. This quality control finally shows that the four species of micropesties are not toxic to the microorganism and do not affect it in its development.

The next check concerns whether the presence of microspheres is affected by precipitation $CaCO_3$ by *St. aureus*.

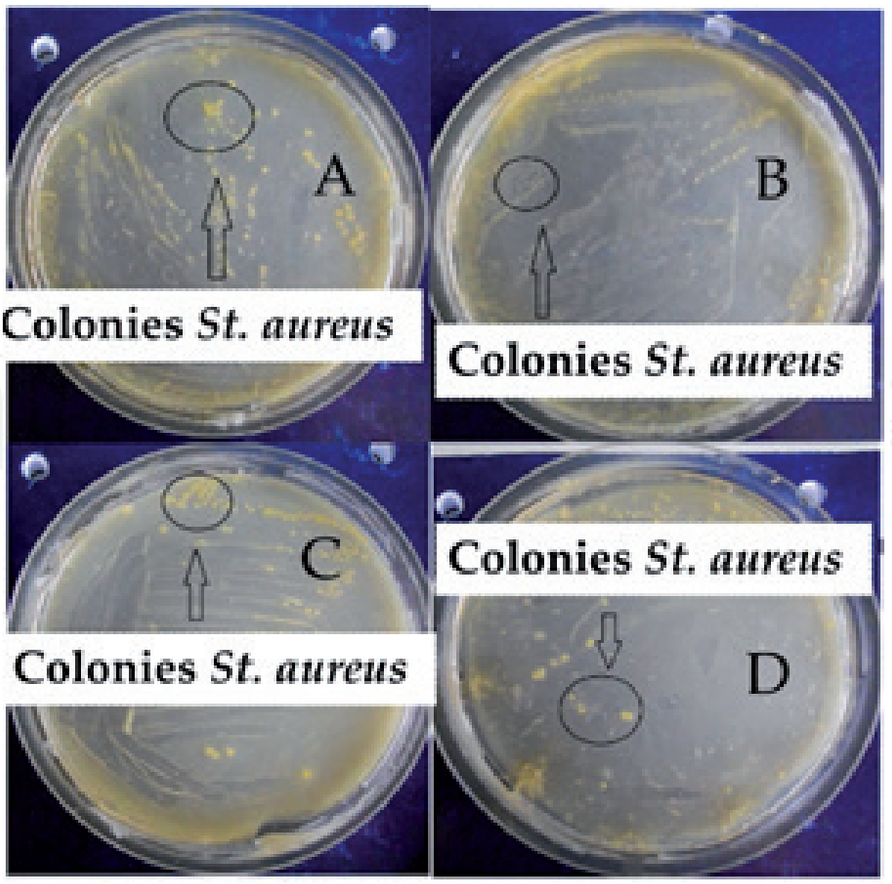

Figure 17.
Development of St. aureus in petri plates in the presence of (A) PMMA microspheres, (B) SiO_2 microspheres, (C) water traps, and (D) SiO_2 coated water microtraps.

3.7.1.3 Precipitation characterization study $CaCO_3$ by Staphylococcus aureus in the presence of microspheres

The effect of microspheres on the precipitation of the $CaCO_3$, from the microorganism, a kinetic medium, was studied in *St. aureus* solutions in the presence of $CaCl_2.2H_2O$, urea, and microspheres. Taking samples at regular intervals, and by characterization of infrared spectroscopy (FT-IR), the existence of the mineral is studied. The microspheres used for this study are water traps.

Table 10 describes the composition of the solutions of the microorganism in a nutrient solution LB for the kinetic precipitation study of $CaCO_3$.

The development of the microorganism in LB nutrient material with the above quantities of materials. In this particular case, however, *St. Aureus*, which develops, is placed in the stirring incubator for about 24 days in certain conditions (37°C, 100 rpm). At regular

Materials	Quantities
LB	49.5 ml
$CaCl_2.2H_2O$	2.5 g
Urea	2.5 g
Solution St. aureus	0.5 ml
Micro-water traps	1 g

Table 10.
Quantities of materials used for the kinetic precipitation study of $CaCO_3$ from microorganism solution to LB nutrient material in the presence of micro-water traps.

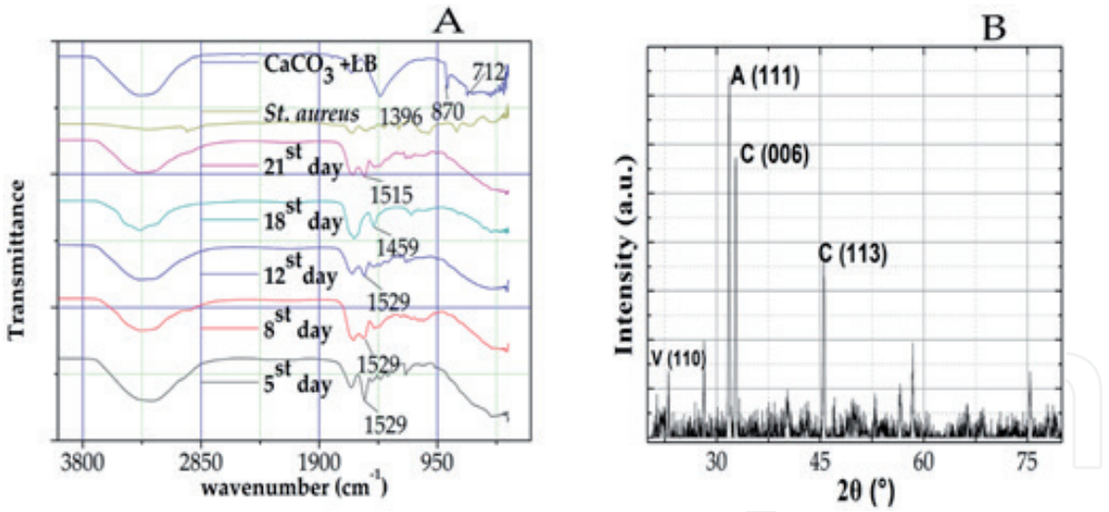

Figure 18.
(A) FT-IR spectrum of $CaCO_3$ kinetic precipitation study by St. aureus in LB nutrient material in the presence of water traps. (B) X-ray radiance spectrum for St. aureus sample in the presence of water microtraps after precipitation of $CaCO_3$.

intervals, a sample is isolated, centrifuged, sterilized, and eventually characterized via FT-IR, for development certification of $CaCO_3$. In other words, in order to identify the carbonic ion peaks in the FT-IR spectra of the isolated samples, a comparative study is carried out between the sample spectra, the microorganism, and the $CaCO_3$ in LB nutrient. It has emerged that $CaCO_3$, in LB solution, has peaks of 712, 873, and 1409 cm^{-1} (**Figure 18A**), while the peaks corresponding to the microorganism are as follows: the peak at 1018 cm^{-1} is attributed to polysaccharides compounds, the peak at 1260 cm^{-1} in the asymmetric vibration of the bond PO_2, at 3274 cm^{-1} we observe the vibration of N-H, while at 2838 cm^{-1}, we observe the symmetrical vibration of the CH_2 bond.

Based on the FT-IR spectrum of **Figure 18**, it appears that $CaCO_3$ is precipitated by *St. aureus* in the LB nutrient material in the presence of water traps. This is demonstrated by the appearance of a peak at 1529 cm^{-1} where it belongs to the carbonate display area 1530–1320 cm^{-1}. The remaining peaks of the sample spectrum are attributed to components of the microorganism itself and the nutrient LB. Further characterization of $CaCO_3$ precipitation by *St. aureus* in the presence of micro water traps was carried out using the XRD method (**Figure 18B**). According to the X-ray spectrum of the sample, the existence of $CaCO_3$ is certified by the coexistence of all three of its crystalline forms: (a) calcite, C with crystalline levels (006) and (113) and (b) baterite, V (110) and aragonite, A (111).

4. Conclusions

The purpose of this work is the synthesis and characterization of an economical and easily manageable material, which will have an impact on microorganisms by attributing to specific conditions of $CaCO_3$, a mineral that allows the healing of cracks in building materials. More specifically, the composition of microspheres of various types such as PMMA has been studied and their interaction with microorganisms, Gram-positive and Gram-negative, which under appropriate conditions cause $CaCO_3$ precipitation. According to the literature, the most appropriate bacterium in the study of such a case is bacillus (Gram positive, alkalophilic, spore-like bacterium). Due to the inability to find bacillus bacterium, the study was carried out on two types of bacteria, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). More specifically, however, St. aureus was investigated as it

is a Gram-positive bacterium. The first system studied is water traps. Microspheres of 1 μm size were made, with a uniform size distribution, and from the water absorption study, it was shown that they can respond to aqueous stimuli by increasing their size due to swelling. Such a system could be used to absorb the seeds of the microorganism. The water traps were then coated with SiO_2 in order to give the water traps a durable shell, so that in the future when added to the concrete, it protects the adsorbed bacterium from the extreme environment of cement. In the second microsphere synthesis system, the development of an organic (PMMA) and an inorganic (SiO_2) transport microsystem is observed. Both types of microspheres were synthesized in aqueous environment, which is chosen by industry for the production of a material, as an economical composition. This contrasts with the composition of the water microtraps and their coatings as the composition was carried out in organic solvent (acetonitrile), which is economically unprofitable for production. The composition of the PMMA and SiO_2 microspheres led to production of approximately 1 μm in size, with a uniform size distribution.

The third system concerns the synthesis of microspheres with polyurea shells for the purpose of encasing a substance inside them. Successful synthesis of urea shell capsules was observed when PEG and Triton-x were used as an emulsifier. This shell has useful properties for the industry, as it has great durability and is stable. In the second part of the work, the ability of *Escherichia coli* and *Staphylococcus aureus* to precipitate $CaCO_3$ was studied. The submersion of the mineral is certified by visual characterization as well as by FT-IR spectroscopy. After it has been shown that microspheres are toxic not only to humans (average MTT method) but also to bacteria (visual reculture control after vaccination in a petri dish), it was studied whether they affect the microorganism's submersion of $CaCO_3$. As observed from the data of this study, microorganism remains metabolically active, which leads to precipitation of the mineral, in the presence of microspheres under specific incubation conditions.

Although the bacterium-sphere interaction mechanism has been fully studied, this study shows that the synthesis of micro-bacteria transport systems for the purpose of self-feeding building materials is a promising way to protect the bacterium within cement. Bibliographically, it has been observed that the viability of seeds of the genus Bacillus within cement decreases over time. Thus, the composition of a material where it can protect the bacterium in such an environment, is promising in the field of self-healing. In this way, the performance of the bacterium in precipitation increases, and more effective healing of the crack will be observed. More objective for the composition of such a material is the resulting product to be as manageable and easy to implement as possible, in order to become industrially competitive.

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